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Association of HIV infection with biomarkers of kidney injury and fibrosis in the Multicenter AIDS Cohort Study

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Abstract

Background—Chronic kidney disease (CKD) is common among HIV-infected individuals, but serum creatinine is insensitive for detecting kidney damage at early stages. We hypothesized that HIV infection would be associated with elevations in subclinical markers of kidney injury and fibrosis in a contemporary cohort of men.

Methods—In this cross-sectional study, we measured urine levels of interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), pro-collagen type III N-terminal pro-peptide (PIIINP), and

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albumin-creatinine ratio (ACR) in 813 HIV-infected and 331 uninfected men enrolled in the Multicenter AIDS Cohort Study.

Results—Median eGFR was 95 ml/min/1.73m² among African-Americans (n=376) and 87 ml/min/1.73m² among Caucasians (n=768). Among HIV-infected men, the median CD4 lymphocyte count was 572 cells/mm³ and 76% of men had undetectable HIV RNA levels. After multivariable adjustment for traditional CKD risk factors including eGFR, HIV infection was associated with 52% higher urine IL-18 (95% CI: 33%, 73%), 44% higher KIM-1 (27%, 64%), 30% higher PIIINP (15%, 47%), and 84% higher ACR (54%, 120%), with similar effect sizes among African-Americans and Caucasians (p>0.2 for tests of interaction by race). These associations remained statistically significant in analyses that excluded persons with detectable HIV RNA levels and in models that adjusted for cumulative exposure to tenofovir disoproxil fumarate.

Conclusions—Compared with uninfected men, HIV-infected men had more extensive glomerular and tubulointerstitial damage, as assessed by urine biomarkers. Future studies should evaluate whether combinations of biomarkers can be used to monitor stages of kidney injury and to predict CKD risk in HIV-infected individuals.

Introduction

In parallel with advances in survival with HIV infection, chronic kidney disease (CKD) has become a common comorbidity.¹ Early in the HIV epidemic, HIV-associated nephropathy^{2–4} was characterized by heavy proteinuria and rapid progression to end-stage renal disease (ESRD). HIVAN almost exclusively affected persons of African descent in whom HIV infection was poorly controlled. By contrast, CKD in the contemporary era of antiretroviral therapy (ART) is frequently observed among individuals with reconstituted immune systems and suppressed HIV viremia. Additionally, the burden of CKD in the HIV-infected population is no longer limited to persons of African descent.^{5–7} Because kidney biopsy is performed in only a subset of individuals, the etiology of CKD is often unknown, and attributed to a variety of clinical risk factors including nephrotoxic antiretroviral therapy, concomitant chronic hepatitis C virus (HCV) infection, and comorbid conditions such as diabetes mellitus and hypertension.^{8–11} Kidney damage is frequently undetected for many years, due to clinical reliance on serum creatinine and proteinuria measurements.¹²

Earlier detection of kidney injury can improve our understanding of HIV-related kidney disease and enable intervention when injury is still reversible. In the Women's Interagency HIV Study (WIHS), we previously found that HIV-infected women had higher urine levels of two novel proximal tubular injury markers, interleukin-18 (IL-18) and kidney injury molecule-1 (KIM-1), as compared with uninfected women, and that urine IL-18 and KIM-1 predicted longitudinal kidney function decline and mortality in HIV-infected participants.^{13–15} We also found that poor HIV control, reflected by lower CD4 lymphocyte counts and higher HIV RNA levels, and HCV coinfection were associated with more extensive proximal tubular injury. However, the WIHS cohort was designed to be representative of the urban HIV epidemic among women in the United States;¹⁶ fewer than one-third of the HIV-infected women in our prior studies were virally suppressed at the time of urine collection in 1999–2000. Consequently, these results may not be generalizable to

the broader HIV-infected population. Additionally, the study was conducted before the widespread use of tenofovir disoproxil fumarate (TDF), which has been associated with Fanconi's syndrome, acute kidney injury, proteinuria and CKD.^{17–19}

The primary objective of this study was to evaluate whether or not HIV infection is associated with kidney injury in a contemporary cohort of men. We performed a cross-sectional study of HIV-infected and uninfected men enrolled in the Multicenter AIDS Cohort Study, evaluating the associations of HIV infection with four urine biomarkers: IL-18 and KIM-1, markers of proximal tubular injury; pro-collagen type III N-terminal pro-peptide (PIIINP), a marker of tubulointerstitial fibrosis; and albumin-creatinine ratio (ACR), a traditional marker of glomerular injury. We also performed race-stratified analyses to evaluate the effects of HIV on kidney injury among African Americans and Caucasians separately, based on established differences in kidney risk.^{6,7,20} Finally, we identified clinical factors associated with higher biomarker levels among HIV-infected participants.

Methods

Study Population and Design

The Multicenter AIDS Cohort Study (MACS) is a prospective cohort study designed to describe the epidemiology and natural history of HIV infection among men who have sex with men. A total of 6,972 HIV-infected and uninfected men were enrolled between 1984 and 2003 from four sites in the United States: Baltimore, Chicago, Los Angeles and Pittsburgh.²¹ Participants attend semiannual visits that include standardized questionnaires, a physical examination, and collection of laboratory specimens.

The MACS Kidney Study was designed as a nested cohort study to investigate the onset and progression of kidney disease among HIV-infected men, using stored urine and serum samples. Urine specimens were refrigerated immediately after collection, and centrifuged at 5000xg to remove cellular debris. The supernatant was aliquoted into 1cc vials and then stored at –80°C until biomarker measurement. A total of 1,234 participants underwent urine collection between October 1, 2009 and September 30, 2011. This cross-sectional study of kidney injury included all 1,144 men who were of self-reported African-American or Caucasian race, and had available urine biomarker measurements from this time period.

The institutional review boards of participating institutions approved the study protocol, and informed consent was obtained from all study participants. This study was also approved by the University of California, San Francisco, San Francisco VA Medical Center, and Johns Hopkins University committees on human research.

Urine Biomarkers

The outcomes of this study were urine levels of four biomarkers: IL-18, KIM-1, PIIINP, and ACR. Biomarker levels were measured at the Cincinnati Children's Hospital Medical Center Biomarker Laboratory. Urine IL-18 was measured using a commercially available ELISA kit (Medical & Biological Laboratories Co., Nagoya, Japan). The urine KIM-1 ELISA was constructed using commercially available reagents (R & D Systems, Inc., Minneapolis, MN).²² Urine PIIINP was measured by a commercially available ELISA (USCN Life

Sciences, Wuhan, Hubei, China). Urine albumin and creatinine were measured by immunoturbidimetry and colorimetric enzyme assay, respectively, using a Siemens Dimension Xpand plus HM clinical analyzer (Siemens, Munich, Germany). Coefficients of variation for the urine measurements were: IL-18, 7.2%; KIM-1, 5.2%; PIIINP, <10%; albumin, 5.9%; and creatinine, 4.1%. Urine specimens were in continuous storage at -80°C until biomarker measurement without prior freeze-thaw. Laboratory personnel performing the biomarker assays were blinded to participants' clinical information.

Covariates

The following demographic and clinical characteristics were tested as candidate covariates in multivariable models: age, race/ethnicity, diabetes mellitus (fasting glucose $\geq 126\text{mg/dL}$; hemoglobin A1c $\geq 6.5\%$; or self-reported history of diabetes and diabetes medication use), systolic and diastolic blood pressure, hypertension (systolic blood pressure $\geq 140\text{ mmHg}$ or diastolic blood pressure $\geq 90\text{ mmHg}$; or self-reported history of hypertension and antihypertensive medication use), cigarette smoking status (current, past, or never), LDL and HDL cholesterol, triglycerides, body mass index (BMI), waist circumference, and hepatitis C virus (HCV) infection (confirmed by detectable HCV RNA following a positive HCV antibody result). Candidate HIV-related characteristics included: current CD4 lymphocyte count, nadir CD4 lymphocyte count, history of clinical AIDS diagnosis,²³ current and peak plasma HIV RNA levels, time-averaged historical HIV RNA level, and cumulative TDF exposure. Glomerular filtration rate was estimated using the CKD-EPI equation for creatinine (eGFR).²⁴ CKD was defined by an $\text{eGFR} < 60\text{ml/min/1.73m}^2$. Multiple imputation with the Markov chain Monte Carlo method was used to impute missing covariates, with 5 imputations to yield $\sim 95\%$ relative efficiency.²⁵

Statistical Analysis

We compared race-stratified demographic and clinical characteristics of HIV-infected and uninfected participants using the Mann-Whitney U and Fisher's exact tests for continuous and categorical variables, respectively. Next, we used multivariable robust regression models with M-estimation and Huber weighting²⁶ to evaluate the associations of HIV infection with each biomarker outcome. Due to their right-skewed distributions, biomarker outcomes were log-transformed to normalize their distributions; results were back-transformed to produce estimated percentage differences. Models were adjusted sequentially for demographics, traditional kidney risk factors, and eGFR. Because urine creatinine is susceptible to bias by muscle mass and health status, we analyzed urine IL-18, KIM-1, and PIIINP without indexing to urine creatinine. In separate analyses, we adjusted for urine creatinine as a covariate to account for urine tonicity, and for ACR to account for clinical evidence of glomerular damage. We also used Poisson relative risk regression with a robust variance estimator²⁷ to model associations of HIV infection with prevalent microalbuminuria, defined using the clinically established ACR threshold of $>30\text{mg/g}$.

To assess for effect modification by race, we stratified analyses by race, and also evaluated interactions of HIV with race for each biomarker outcome. To evaluate the effects of HIV infection on kidney injury in the absence of clinical CKD, sensitivity analyses were restricted to the 1046 individuals with $\text{eGFR} > 60\text{ml/min/1.73m}^2$. We also excluded persons

with detectable HIV RNA levels at the time of urine biomarker measurement (n=205), in order to evaluate whether HIV infection is associated with biomarker levels despite suppressed viremia.

Finally, we performed analyses to compare and quantify associations of demographics, traditional and HIV-related risk factors with each biomarker among HIV-infected participants. We adjusted for cumulative TDF exposure in the multivariable models, based on known clinical associations with nephrotoxicity.^{9,18,28} Associations of TDF and other antiretroviral medications with kidney injury biomarkers are described separately in this cohort.²⁹ We used stepwise backward selection with a significance level of $\alpha=0.05$ to remove candidate covariates that were not associated with outcome measures. As an alternative model building approach, we used Bayesian model averaging and retained predictors with posterior probabilities >35%.³⁰ Models constructed using the two approaches were very similar.

Bayesian model averaging was performed using the BMA package for the R statistical computing language (R Development Core Team, Vienna, Austria). All other analyses were conducted using the SAS system, version 9.4 (SAS Institute, Inc., Cary, NC).

Results

Study population by race and HIV status

Median age was 52 among the 813 HIV-infected participants and 55 among the 331 uninfected participants. Approximately one-third of the HIV-infected (n=274) and uninfected (n=102) participants were African-American (Table 1). Diabetes mellitus and hypertension were prevalent in approximately one-sixth and one-half of participants, respectively, and three-quarters of participants were current or former smokers. HCV coinfection was far more common among African-Americans (23%) than in Caucasians (4%). The median eGFR was 95 mL/min/1.73 m² in African-Americans and 87 in Caucasians, and showed little difference by HIV serostatus within each racial group.

Among the HIV-infected participants, median CD4 lymphocyte counts were 560 and 580 cells/mm³ in African-Americans and Caucasians, respectively; HIV RNA levels were undetectable in 64% of African-Americans and 88% of Caucasians. The prevalence of TDF use among HIV-infected participants was 63% at the time of urine biomarker measurement, and did not differ by race. Median duration of TDF exposure was 2.3 years in African-Americans and 3.4 years in Caucasians.

Associations of HIV infection with kidney injury biomarkers

Median urine levels of IL-18, KIM-1, PIIINP, and ACR were higher among HIV-infected participants, compared with uninfected participants, among both African-American and Caucasian men (Figure 1). After controlling for traditional kidney risk factors including eGFR (Table 2), HIV infection was associated with 52% higher IL-18, 44% higher KIM-1, 30% higher PIIINP, and 84% higher ACR. HIV infection remained independently associated with higher urine IL-18, KIM-1, and PIIINP levels after additional adjustment for ACR and urine creatinine levels, although the effect sizes were moderately attenuated.

Adjustment for cumulative TDF exposure moderately attenuated the associations of HIV infection with biomarker levels, but the associations remained statistically significant. In models adjusting for demographics, traditional kidney risk factors, and cumulative TDF exposure, HIV was associated with 42% higher urine IL-18 (95% CI: 22, 65; $p<0.001$), 33% higher KIM-1 (95% CI: 15, 55; $p<0.001$), 19% higher PIIINP (95% CI: 3, 37; $p=0.015$), and 65% higher ACR (95% CI: 35, 102; $p<0.001$).

In race-stratified analyses, HIV infection remained strongly associated with higher biomarker levels in both African-Americans and Caucasians. There were no statistically significant interactions between HIV serostatus and race for the biomarker outcomes ($p>0.2$ for tests of interaction). Effect sizes were similar when we restricted analyses to participants with $eGFR>60\text{ml/min/1.73m}^2$ (Supplemental Table 1), and when we excluded HIV-infected participants with detectable HIV RNA levels (Supplemental Table 2).

The prevalence of albuminuria ($ACR>30\text{ mg/g}$) was higher among HIV-infected participants, compared with uninfected participants, in both African-Americans (23% vs. 13%, respectively) and Caucasians (16% vs. 6%, respectively). After adjustment for traditional kidney risk factors, HIV infection was associated with a 2-fold prevalence of albuminuria among African-Americans (Prevalence ratio=2.1; 95% CI: 1.2, 3.5) and among Caucasians (Prevalence ratio=2.5; 95% CI: 1.4, 4.3).

Factors associated with kidney injury biomarkers among HIV-infected men

We then constructed multivariable regression models to identify factors associated with kidney injury biomarkers in HIV-infected men (Table 3). Older age (per decade) was associated with higher urine levels of all four biomarkers, with the largest effect size seen for ACR. African-American race was associated with higher urine levels of IL-18, PIIINP, and ACR, but not with urine KIM-1. Among HIV-related characteristics, lower CD4 lymphocyte count and HCV coinfection were independently associated with higher levels of all four biomarkers. Additionally, higher HIV RNA and lower HDL levels were associated with higher urine IL-18, and larger waist circumference was associated with higher urine KIM-1. Higher systolic blood pressure, antihypertensive use, higher triglycerides, and current smoking were each independently associated with higher ACR.

Discussion

In this cohort of HIV-infected and uninfected men who have sex with men, HIV infection was associated with higher urine levels of IL-18, KIM-1, PIIINP, and ACR among African-Americans and Caucasians, independent of traditional risk factors for kidney disease and $eGFR$. These associations remained statistically significant after adjustment for TDF exposure. Among the HIV-infected men in this cohort, predictors of higher levels of all four biomarkers included older age, lower CD4 lymphocyte count, and HCV infection. To our knowledge, this is the first study to evaluate the associations of HIV infection with these specific biomarkers of kidney damage in a large, contemporary cohort of men. In combination with our earlier work demonstrating associations of urine IL-18 and KIM-1 with longitudinal kidney function decline and mortality among HIV-infected women,^{13,14} these findings support an ongoing role for HIV infection in the development of kidney

injury, even among virally suppressed persons who are receiving effective antiretroviral therapy.

In contrast to albuminuria, which is a clinical marker of glomerular injury, urine IL-18, KIM-1, and PIIINP are novel biomarkers of tubulointerstitial injury and fibrosis. Originally identified as markers of acute kidney injury, IL-18 and KIM-1 are released into the urine by injured proximal tubular epithelial cells, with levels rising by several logs of magnitude in the setting of ischemic acute tubular necrosis.^{31,32} By contrast, PIIINP (the amino-terminal pro-peptide of type III collagen) is released into urine during deposition of type III collagen in the extracellular matrix;³³ therefore, urine PIIINP is indicative of ongoing renal fibrotic processes. In kidney biopsy series, higher urine PIIINP levels were associated with the severity of tubulointerstitial fibrosis.^{34,35} Additionally, Ix *et al.* recently found that urine PIIINP levels were independently associated with faster CKD progression in a cohort of elderly individuals.³⁶ Taken together, our observed associations between HIV infection and higher urine IL-18, KIM-1, and PIIINP suggest that HIV infection may promote tubulointerstitial injury and fibrosis.

In the setting of HIV infection, tubulointerstitial damage may occur via several potential mechanisms. First, the occurrence of direct toxicity to the renal tubular epithelium by HIV-1 is supported by prior studies demonstrating the presence of HIV-1 in the renal tubular epithelium of human kidney biopsy specimens and transgenic murine models of HIV-associated nephropathy.^{37–40} Furthermore, there have been reports of HIV-infected individuals with undetectable plasma HIV RNA, in whom HIV-1 RNA and DNA remain detectable in renal tubular epithelial cells.^{37,38} In addition to implicating the kidney as a reservoir for HIV-1, these observations raise the possibility that the virus continues to exert toxicity to tubular epithelial cells during periods of suppressed viremia. Second, nephrotoxicity from antiretroviral therapy can occur through multiple mechanisms including direct tubular toxicity, acute interstitial nephritis, and crystal-induced obstruction. We recently reported that cumulative TDF exposure was associated with higher urine levels of IL-18, KIM-1, and PIIINP.²⁹ This finding is consistent with prior literature implicating the proximal tubular epithelium as the primary site of TDF-associated injury.^{17,41,42} Finally, recent studies suggest a synergistic effect of HIV/HCV coinfection on kidney injury.^{10,11,43} A meta-analysis of 12 clinical trials and observational studies reported that HIV/HCV coinfection was associated with a 50% increased risk of incident CKD and a 20% increased risk of proteinuria.¹¹ Further studies are needed to elucidate the pathophysiological basis for these findings, and to determine whether HCV treatment will mitigate longitudinal kidney risk in coinfecting individuals.

An important finding of this study is that HIV infection is associated with higher biomarker levels among both African-Americans and Caucasians. Large cohort studies of HIV-infected individuals have shown that African-Americans have substantially elevated incidence of ESRD, when compared with Caucasians, as well as faster progression from CKD to ESRD.^{6,7,20} Recent genetic studies have determined that approximately 10–15% of African-Americans carry specific high-risk variants on the *APOL1* gene, which lead to elevated risks for kidney disease.^{44–46} In a study of HIV-infected African-American women, we reported that the high-risk *APOL1* genotype was associated with a 2-fold risk of albuminuria and 1.7-

fold risk of incident CKD over 8 years.⁴⁷ Consistent with these epidemiologic and genetic studies, we found that HIV-infected African-American men had higher urine levels of IL-18, PIIINP, and ACR, compared with HIV-infected Caucasians. However, in race-stratified analyses, HIV infection was associated with substantially elevated levels of all four biomarkers in Caucasians and African-Americans, suggesting that both racial groups are susceptible to kidney damage in HIV.

This study has important implications. First, despite the relatively high prevalence of individuals with suppressed HIV in this cohort, our findings raise the possibility that kidney injury and fibrosis may be ongoing in HIV-infected individuals. Future studies must correlate these and other promising urine biomarkers with kidney biopsy specimens, to better understand the underlying mechanisms of kidney injury in this population. Second, the broad spectrum of HIV-related kidney disease offers a platform for the identification of biomarker injury patterns that can guide clinical decision-making. For instance, specific combinations of biomarkers may enable clinicians to distinguish drug-induced nephrotoxicity from other etiologies of kidney damage, and thereby avoid changes to antiretroviral regimens that are not indicated. Finally, improved kidney diagnostics must occur in parallel with rigorous evaluation of interventions that slow the progression of kidney disease among HIV-infected individuals, such as blood pressure control and renin-angiotensin system blockade.

There are several limitations to this study. First, the cross-sectional design limits our ability to infer causality for the observed associations. Second, this study was limited to men. However, our earlier work in the WIHS cohort revealed that HIV-infected women also had more extensive kidney injury, relative to uninfected women. Third, we did not have access to serum levels of IL-18, KIM-1 and PIIINP. Although IL-18, KIM-1 and PIIINP are not known to be filtered or secreted by the kidney, we cannot exclude the possibility that higher serum levels contributed to our observations. Fourth, this study was not designed to evaluate the potential renoprotective effects of renin-angiotensin system (RAS) blockade. Future longitudinal cohort studies and clinical trials are needed to determine whether RAS inhibitors are effective treatments for kidney injury in HIV-infected individuals. Finally, despite adjustment for multiple potential confounders, the possibility of residual confounding exists for the associations of HIV infection with the kidney injury biomarkers.

In conclusion, HIV-infected men had more extensive glomerular and tubulointerstitial kidney damage than uninfected men, as assessed by urine biomarker levels. Future studies should investigate the utility of screening HIV-infected individuals with a urine biomarker panel, in order to identify kidney injury at earlier stages and, ultimately, reduce kidney disease burden in this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Urine biomarker levels by HIV serostatus in African American and Caucasian MACS participants

Table 1

Characteristics of MACS participants, stratified by race and HIV serostatus

	African American (n=376)			Caucasian (n=768)		
	HIV-infected (N = 274)	Uninfected (N = 102)	P-value	HIV-infected (N = 539)	Uninfected (N = 229)	P-value
Age (y)	50 (45–55)	50 (47–55)	0.13	54 (49–59)	57 (52–63)	<0.001
Diabetes mellitus	19%	26%	0.16	15%	10%	0.11
Systolic BP (mm Hg)	126 (117–134)	128 (115–136)	0.56	127 (117–136)	128 (117–138)	0.29
Diastolic BP (mm Hg)	79 (72–85)	79 (71–86)	0.75	78 (72–84)	78 (72–84)	0.67
Hypertension	43%	47%	0.48	51%	50%	0.80
Antihypertensive use	33%	32%	0.93	37%	36%	0.76
Hepatitis C	21%	28%	0.14	5%	1%	0.024
Cigarette smoking						
Current	44%	51%	0.17	23%	14%	0.022
Past	34%	36%		50%	56%	
Never	22%	13%		27%	30%	
LDL (mg/dL)	106 (84–129)	107 (89–126)	0.87	108 (89–133)	118 (96–139)	0.002
HDL (mg/dL)	48 (39–59)	49 (41–60)	0.55	45 (38–53)	50 (41–60)	<0.001
TG (mg/dL)	112 (82–172)	100 (68–138)	0.021	150 (103–225)	114 (77–164)	<0.001
Body Mass Index (kg/m²)	26 (23–31)	29 (26–36)	0.001	26 (23–30)	26 (24–31)	0.23
Waist Circumference (cm)	91 (83–102)	96 (88–110)	0.002	95 (88–103)	97 (89–106)	0.007
eGFR_{Cr} (ml/min/1.73m²)	95 (80–114)	93 (84–112)	0.77	86 (73–98)	87 (76–96)	0.76
eGFR_{Cr} <60ml/min/1.73m²	7%	5%	0.57	10%	4%	0.002
Current CD4 (cells/mm³)	560 (374–719)			580 (422–753)		
Nadir CD4 (cells/mm³)	298 (189–429)			285 (174–397)		
History of AIDS	10%			17%		
Current HIV Viral Load (copies/mL)						
<80	64%			88%		
80–2000	16%			6%		
2000–9999	6%			2%		
>10000	14%			4%		
HAART use	76%			89%		

	African American (n=376)		Caucasian (n=768)	
	HIV-infected (N = 274)	Uninfected (N = 102)	HIV-infected (N = 539)	Uninfected (N = 229)
				P-value
NRTI use	78%		88%	
NNRTI use	36%		54%	
PI use	45%		43%	
TDF use	63%		65%	

Continuous data are presented as Median (IQR).

Abbreviations: HAART, highly active antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate.

Table 2

Associations of HIV infection with biomarker levels among MACS participants, overall and stratified by race

Population Model	IL-18		KIM-1		PIINP		ACR	
	% Estimate ¹	(95% CI)	P Value	% Estimate (95% CI)	P Value	% Estimate (95% CI)	P Value	P Value
Overall (n=1144)								
Demographic-adjusted ²	51 (33, 72)		<0.001	41 (24, 60)	<0.001	31 (16, 47)	<0.001	98 (67, 134)
Multivariable-adjusted+eGFR ³	52 (33, 73)		<0.001	44 (27, 64)	<0.001	30 (15, 47)	<0.001	84 (54, 120)
Multivariable-adjusted+eGFR +ACR+U _{Cr} ⁴	29 (16, 45)		<0.001	27 (14, 41)	<0.001	9 (0.1, 20)	0.047	--
African Americans (n=376)								
Demographic-adjusted ²	52 (21, 92)		<0.001	27 (1, 60)	0.038	30 (6, 60)	0.012	57 (16, 111)
Multivariable-adjusted+eGFR ³	36 (10, 69)		0.005	35 (8, 70)	0.010	34 (8, 66)	0.007	70 (25, 132)
Multivariable-adjusted+eGFR +ACR+U _{Cr} ⁴	42 (17, 73)		<0.001	21 (1, 46)	0.042	12 (-5, 30)	0.17	--
Caucasians (n=768)								
Demographic-adjusted ²	51 (29, 76)		<0.001	48 (27, 73)	<0.001	30 (13, 50)	<0.001	120 (80, 170)
Multivariable-adjusted+eGFR ³	29 (11, 49)		<0.001	49 (27, 74)	<0.001	28 (11, 49)	<0.001	91 (54, 136)
Multivariable-adjusted+eGFR +ACR+U _{Cr} ⁴	24 (8, 41)		0.002	29 (14, 47)	<0.001	9 (-3, 21)	0.14	--
P-value for HIV*race interaction	0.23			0.58		0.77		0.55

¹ Estimated percentage difference in biomarker attributable to HIV infection² Adjusted for age and race³ Adjusted for age, race, diabetes mellitus, hypertension, anti-hypertensive medication use, cardiovascular disease, heroin use, and hepatitis C virus infection, and eGFR⁴ Adjusted for above factors, albumin-creatinine ratio, and urine creatinineAbbreviations: ACR, albumin-creatinine ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; PIINP, procollagen type III amino-terminal pro-peptide; U_{Cr}, urine creatinine.

Table 3
Multivariable adjusted associations with urine biomarker concentrations among HIV-infected MACS participants (N=813)

Parameter	IL-18	KIM-1	PIIINP	ACR
	% Estimate [†] (95% CI)	% Estimate (95% CI)	% Estimate (95% CI)	% Estimate (95% CI)
Age (per decade)	13 (4, 22) **	25 (15, 35) **	18 (9, 27) ***	35 (20, 52) ***
African-American vs. Caucasian	54 (32, 79) ***	-9 (-21, 6)	17 (2, 36) *	36 (9, 69) **
CD4 Count (per doubling)	-15 (-22, -7) ***	-14 (-20, -6) ***	-14 (-20, -6) ***	-25 (-33, -15) ***
Hepatitis C infection	39 (11, 74) **	33 (7, 65) *	35 (9, 68) **	69 (23, 133) **
HIV viral load (per 10-fold increase)	22 (13, 31) ***	-	-	-
HDL (per 10 mg/dL)	-6 (-10, -2) **	-	-	-
Waist circumference (per 10 cm)	-	7 (1, 14) *	-	-
SBP (per 10 mmHg)	-	-	-	17 (10, 25) ***
Antihypertensive use	-	-	-	45 (18, 79) ***
Triglycerides (per doubling)	-	-	-	33 (18, 49) ***
Current smoking	-	-	-	41 (14, 74) **

Models adjust for factors displayed beneath each biomarker and cumulative TDF exposure

[†] Estimated percentage difference in biomarker attributable to each factor

*, **, *** denote P-value < .05, < .01, < .001, respectively

Abbreviations: ACR, albumin-to-creatinine ratio; HDL, high-density lipoprotein; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; PIIINP, procollagen type III amino-terminal pro-peptide; SBP, systolic blood pressure.